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(54) Title: MONOCLONAL ANTIBODIES SPECIFIC FOR ANTHRAX SPORES AND PEPTIDES DERIVED FROM THE ANTIBODIES THEREOF

(57) Abstract

The present invention provides monoclonal antibodies which are highly specific for *Bacillus* spores. Also provided are peptides derived from those monoclonal antibodies. Both the antibodies and peptides are highly specific and can discriminate between spores of potentially lethal organisms such as *Bacillus anthracis* and other harmless but closely related bacilli and provide a very powerful tool in the construction of detection instruments as counter measures.

16, + 18 102

17-20 103 w/ colloidal

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MONOCLONAL ANTIBODIES SPECIFIC FOR ANTHRAX SPORES AND PEPTIDES DERIVED FROM THE ANTIBODIES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to the fields of immunology and microbiology. More specifically, the present invention relates to monoclonal antibodies specific for anthrax spores and peptides derived from the antibodies.

Description of the Related Art

During the evolution of the immune system there is evidence that the repertoire of germline V genes that has been retained in the genome has been subject to selective processes by

environmental influences which may include self as well as commensal and non-commensal microorganisms. Structural and functional analysis of immunoglobulin and T cell receptors have delineated regions of these molecules which are germline encoded and have the ability to bind to certain bacterial components through exposed parts of the molecules which do not need somatic diversification for expression of the ability to bind to these structures. Some of these included protein A binding to framework three (FR3) region of V_H genes, staphylococcal enterotoxin binding to T cell receptor, etc.

The prior art is deficient in the lack of monoclonal antibodies which are highly specific and can discriminate between spores of the Bacillus family including the strategically important *B. anthracis*. Further, the prior art is deficient in the lack of peptides derived from the monoclonal antibodies highly specific for Bacillus spores. The present invention fulfills this longstanding need and desire in the art.

SUMMARY OF THE INVENTION

The present invention is directed to monoclonal antibodies which are highly specific for anthrax spores and peptides derived

from the antibodies. The present invention demonstrated that the humoral immune response to spores of *Bacillus* show a remarkable conservation of V_H gene usage which is distinct for each spore analyzed. The results imply evolutionary conservation of V_H genes due to their ability to bind spores. Furthermore, of highly practical importance, these antibodies can discriminate between spores of potentially lethal organisms such as *B. anthracis* and other harmless but closely related bacilli and provide a very powerful tool in the construction of detection instruments as counter measures in biological warfare.

In one embodiment of the present invention, there is provided a monoclonal antibody specific for *Bacillus* spores. Preferably, *Bacillus* is selected from the group consisting of *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus subtilis* and other bacilli related to *Bacillus anthracis*. Preferably, the antibody is IgG.

In another embodiment of the present invention, there is provided a peptide derived from the monoclonal antibody highly specific for *Bacillus* spores.

In yet another embodiment of the present invention, there is provided a method of preparing the monoclonal antibody highly specific for *Bacillus* spores by immunizing and fusing local lymph nodes of an animal.

In still yet another embodiment of the present invention, there is provided a method of detecting *Bacillus* spores in a field sample using a monoclonal antibody highly specific for the *Bacillus* spores by contacting the sample with a monoclonal antibody disclosed herein.

In still yet another embodiment of the present invention, there is provided a method of detecting *Bacillus* spores in a field sample using a peptide derived from a monoclonal antibody highly specific for the *Bacillus* spores by contacting the sample with a peptide derived from a monoclonal antibody highly specific for *Bacillus* spores.

Other and further aspects, features, and advantages of the present invention will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

So that the matter in which the above-recited features. advantages and objects of the invention, as well as others which will become clear. are attained and can be understood in detail, more particular descriptions of the invention briefly summarized above

may be had by reference to certain embodiments thereof which are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate preferred embodiments of the invention and therefore are not to be considered limiting in their scope.

Figure 1 shows antibodies with different reactivities for germinated and ungerminated spores among different clones.

Figure 2 shows that anti-spore monoclonal antibodies do not react with vegetative bacteria.

Figure 3 shows that most antibodies react specifically with spores of *Bacillus subtilis*.

Figure 4 shows that a commonly used member V_H7183.6 heavy chain gene of the V_H7183 (MOPC21) family in all hybridomas reactive with *Bacillus subtilis* spores appears to be the most unique member of this family in the framework three (FR3) region. (Hybridomas g07 to f10 are labeled SEQ ID NO: 7 to SEQ ID NO: 17, respectively)

Figure 5 shows that antibody-derived peptides specifically bind *Bacillus subtilis* spores.

Figure 6 shows that anti-*Bacillus anthracis* antibody specifically bind *Bacillus anthracis* spores.

Figure 7 shows that the V_H gene sequences among

monoclonal antibodies to *Bacillus anthracis*. (SEQ ID NO: 18 to SEQ ID NO: 31)

Figure 8 shows the discriminatory ability of the antibodies *in vitro*. *Bacillus anthracis*, *Bacillus subtilis* and *Bacillus thuringiensis* spores were mixed and stained on a slide with fluorescent antibodies labeled with blue, green and red antibodies, respectively.

Figure 9 shows the discriminatory ability of the antibodies *in vivo*. A section of mouse spleen was injected 30 minutes previously with *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus subtilis* spores and labeled with blue, green and red antibodies, respectively.

DETAILED DESCRIPTION OF THE INVENTION

In the present invention, panels of monoclonal antibodies which are highly specific and can discriminate between spores of the *Bacillus* family including the strategically important *Bacillus anthracis* (anthrax) were isolated and characterized. The amino acid sequences of these anti-spore antibodies were determined from the nucleotide sequences of the coding genes and smaller peptide molecules were derived from these antibodies which can also bind *Bacillus* spores.

The present invention is directed to a monoclonal antibody highly specific for *Bacillus* spores. Preferably, *Bacillus* is selected from the group consisting of *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus subtilis* and other bacilli closely related to *Bacillus anthracis*. Preferably, the antibody is IgG. The present invention is also directed to a peptide derived from the monoclonal antibody highly specific for *Bacillus* spores. Preferably, the peptide can also bind the *Bacillus* spores specifically.

Also disclosed in the present invention is a method of preparing a monoclonal antibody highly specific for *Bacillus* spores, comprising the steps of immunizing an animal with the *Bacillus* spores and fusing local lymph nodes of the animal.

The present invention also is directed to a method of detecting anthrax in a field sample using a monoclonal antibody highly specific for *Bacillus anthracis* spores by contacting the sample with the monoclonal antibody and measuring the amount of binding of the antibody to the sample compared to an appropriate control.

The present invention also is directed to a method of detecting lethal *Bacillus* spores in a field sample using the peptide derived from the monoclonal antibody highly specific for *Bacillus* spores by contacting the sample with the peptide and measuring the amount of binding of the peptide to the sample compared to an

appropriate control.

The following terms have the definitions set below.

As used herein, "hybridoma" refers to a continuously growing antibody-secreting cell line derived from the fusion of a specific normal antibody-forming B cell from an immunized mouse with an immortal myeloma cell line. Hybridomas secrete monoclonal antibodies described herein.

As used herein, "homogeneous staining" refers to the uniform staining at a similar intensity of all spores in a given sample.

As used herein, "heterogeneous staining" refers to the staining of one or more populations of spores in a given sample.

As used herein, "unfixed untreated spores" refers to spores that are in their native state in water and not treated with any kind of fixation reagent such as formalin or glutaraldehyde or paraformaldehyde.

As used herein, "two-color flow cytometric analysis" refers to the identification of spore subpopulations or other particles by fluorescence activated flow cytometry using two independent fluorochrome labeled antibodies.

As used herein, "limiting dilution" refers to the distributing of hybridoma cells into tissue culture plates such that less than 30% of the wells contain a growing clone. Each well, according to

the Poisson distribution, should contain the progeny of only one cell.

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion.

EXAMPLE 1

Animals

Eight to twelve-week-old BALB/c mice were purchased from Charles River Laboratories (Raleigh, NC) or bred in our mouse facility. BALB/c mice were used for immunization and obtaining feeder cells for subcloning of hybridomas, phenotypic analysis and functional studies. Mice were housed in accordance with institutional policies for animal care and usage.

EXAMPLE 2

Bacterial Spores

B. subtilis spores were provided by Dr. Chuck Turnbough. *B. anthracis* spores were obtained from Dr. Joany J. Jackman at USAMIRID and *B. thuringiensis* spores were obtained from Abbott Laboratories.

EXAMPLE 3

Antibody Production: Immunization and Fusion

Six-week-old female BALB/c mice were inoculated with $5X10^8$ spores emulsified in complete Freund's adjuvant at day 0, and then repeatedly with spores in saline at days 3, 6, 9, 13, 17 and 20 in subcutaneous sites in the rear legs and inguinal regions.

On day 21, popliteal, inguinal and iliac lymph nodes draining sites of injection were removed, a lymphocyte suspension was prepared and fused to P3x63Ag8.653 using a modification of the method described by Kohler and Milstein. Fused cells were plated on ten 96-well plates in DMEM supplemented with 20% fetal bovine serum (FBS), 2 mmol/L L-glutamine, HAT medium, and FCS (from HyClone Laboratories Inc., Logan, UT; other reagents from Sigma), and placed in a 37°C incubator with 9% CO_2.

EXAMPLE 4

Primary Screening and Subcloning

Hybridoma supernatants were screened on spore suspensions using two-color flow cytometric analysis. Binding of secreted mouse Ig from supernatants to the spores was traced with

phycoerythrin (PE)-conjugated goat anti-mouse Ig (Southern Biotechnology Associates, Birmingham, AL). Data from stained cell samples were acquired using a FACScan or FACSCalibur flow cytometer with lysis II and Cell Quest software packages (Becton Dickinson, Mountain View, CA) and analyzed with WinList 2.01 (Verity Software House, Inc.) and WinMDI 2.0 software programs (Trotter@scripps.edu).

EXAMPLE 5

ELISA

Flat bottom ELISA plates (E.I.A.A/2 plates, Costar) were coated with poly-L-Lysine (50 μg) for 30 minutes and a suspension of spores at 2×10^8/ml (40 μl) in distilled water were allowed to dry on the plates overnight. Supernatants were added and after incubation developed with goat anti-mouse Ig. Between each step, the plate was washed five times with PBS. The plate was developed with alkaline phosphatase substrate (Sigma, St. Louis, MO) (1 mg/ml) in substrate buffer (pH 9). For quantitative ELISA, mouse antibody of known concentration was used as a standard in each plate and OD_{405} values of plates were read by a Titertek Multiskan Plus MKII spectrophotometer (Flow, McLean, VA). Antibody concentration was

determined using an ELISALITE program (Meddata, New York, NY).

EXAMPLE 6

Antibody Purification and Conjugation

Pure anti-spore antibodies were prepared from bulk hybridoma cultures by protein G chromatography. FITC and phycoerythrin conjugates were prepared using standard procedures.

EXAMPLE 7

Immunofluorescence and Immunohistochemical Analysis of Tissue Sections and Cytocentrifuge Preparations

Spleens embedded in OCT compound (Lab-Tek Products, Naperville, IL) were flash frozen in liquid nitrogen. Frozen sections were cut, air dried, fixed in ice-cold acetone, blocked with normal horse serum. and macrophages stained with MOMA-1 (rat, IgG2a, 10 μg/ml, from Dr. Georg Kraal), each developed with biotin-conjugated goat anti-rat IgG (SBA). Next, the sections were blocked with normal rat serum followed by anti-spore reagents and secondary reagents and streptavidin AMCA (Vector Laboratories, Burlingame, CA). Spore suspension in distilled water were dryed onto poly-L-Lysine treated

glass slides for 2 hours at 37°C, blocked with 1% BSA and PBS and stained with antibodies for microscopy of spore suspensions.

Tissue sections and slides with dried spores were washed and mounted in Fluormount G (SBA, Birmingham, AL) and viewed with a Leica/Leitz DMRB fluorescence microscope equipped with appropriate filter cubes (Chromatechnology, Battleboro, VT). Images were acquired with a C5810 series digital color camera (Hamamatsu Photonic System, Bridgewater, NJ) and processed with Adobe Photo Shop and IP LAB Spectrum software (Signal Analytics Software, Vienna, VA).

EXAMPLE 8

DNA Sequencing Analysis

V_H and $V\kappa$ gene sequencing was carried out from cDNA isolated from hybridomas. To make cDNA, total RNA was isolated from hybridomas using guanidinium thiocyanate-phenol-chloroform extraction. The cDNA was synthesized using an oligo-dT primer followed by PCR using a $C\mu$ 3' primer (SEQ ID NO: 1) and V_H7183-specific primer (SEQ ID NO: 2) for the heavy chains or a $C\kappa$ 3' primer (SEQ ID NO: 3) and a degenerate $V\kappa$ 5' primer (SEQ ID NO: 4) for the light chains. The PCR amplified DNA was cloned into

Bluescript II KS and subjected to sequencing using a Sequenase Kit (Strategene, La Jolla, CA). The DNA sequences were analyzed using the DNAstar program.

EXAMPLE 9

Antibodies to *B. subtilis*

Two immunization were made, one with fixed spores which gave only 3/192 (1.6%) monoclonal antibodies (mAbs) reactive with spores; and the other with unfixed spores which gave 95/384 (25%) (mAbs) reactive with spores, another 89 (20%) weakly reactive. These 576 clones were then tested against other spore components and 15 reacted with NAD synthetase, 6 with RNA polymerase, 5 with cot TC, 2 with SSPC, and 1 with cse60 by ELISA. Totally, 136 clones were reactive with spores or purified/recombinant components.

Among the clones reactive with the intact spores, certain patterns were observed: (1) two clones appeared to dramatically alter the FSC/SSC profile of spores on flow cytometry; (2) some clones reacted with germinated, but not with ungerminated spores; and (3) the majority had homogenous staining of germinated, but heterogeneous staining of ungerminated spores (Figure 1).

96 clones of hybridomas reactive with *B. subtilis* were

picked up and grown on a new plate. They include two negative clones, the clones reactive with purified proteins or peptides and clones reactive with spores representing different patterns. These antibodies were tested on the vegetative forms of *B. subtilis* (i.e., live bacteria) and were found to be negative (Figure 2). They also did not react with two other species of spore-forming *Bacilli* (Figure 3). Isotyping of the antibodies produced by these clones revealed that many (55/96) use λ light chains. Additionally, it was also unusual that 4 of these antibodies use α heavy chains.

All 96 clones were subcloned by limiting dilution and tested by flow cytometry. 68/96 were still reactive with spores and all except one were monoclonal. The reactive clones can be basically separated into two groups: those reactive with all spores and the other reactive with subsets of spores. Since these antibodies are of different isotypes, multiple parametric flow cytometric analysis could be done next. These important results showed that fixation of spores did not permit production of antibodies to the intact native spores and it was only when unfixed untreated spores were used to immunize mice could many highly specific antibodies to *B. subtilis* be isolated.

EXAMPLE 10

Sequence Analysis of Monoclonal Antibody to *B. subtilis*

The striking over usage of λ light chains in the antibodies led to sequencing the heavy and light chains of the genes from hybridomas synthesizing the *B. subtilis* spore specific antibodies to obtain an idea of the heterogeneity of antibodies generated. The heavy chains revealed remarkable homogeneity of V_H gene usage in that all hybridomas used a member of V_H7183 (MOPC21) family. This member V_H7183.6 appears to be the most unique member of this family in the framework three (FR3) region as shown in Figure 4. The CDR3 region was diverse in nearly all cases and used variable D_H and J_H genes. These results suggest that there is a very strong selection for the use of this V_H gene despite the similarities inherent in the family members of this family. λ light chain sequence showed the exclusive use of Vλ1 Cλ1 with different CDR3 regions. Six of these were selected for further study and characterization.

EXAMPLE 11

Isolation of FR3 Peptides Which Bind to Spores

Based on the sequences of VH genes utilized in

antibodies against *Bacillus subtilis* spores, two peptides were designed: one corresponding to the consensus sequence of these antibodies in the framework 3 region (Peptide Anti-spore: SEQ ID NO: 5), and the other corresponding to the consensus sequences of the 7183 V_H gene family to which the particular V_H gene belongs (Peptide 7183 consensus: SEQ ID NO: 6).

The carboxyl-terminal cysteine was added for fluorochrome conjugation. Both peptides were conjugated with phycoerythrin, and tested for their ability to bind *Bacillus subtilis* spores. 7183 consensus peptide was designed to be a control. It was found that the peptides derived from the anti-spore antibody stained brightly at 2 μg/ml (1 μM), while the consensus peptide stained spores at 200 μg/ml (100 μM) (Figure 5). Thus the peptide derived from the spore specific nucleotide derived antibody sequence bind strongly and specifically to *B. subtilis*.

EXAMPLE 12

Serum Antibody Response to *B. subtilis* Spore Immunization

The immune response to *Bacillus subtilis* spores was characterized in mice. BALB/c mice were immunized with either spores or PBS (control). The mice were bled at 1, 2 and 3 weeks after

immunization. Serum antibodies of different isotypes specific for spores were quantitated using ELISA. It was found that (1) immune responses peaked at 1 week; (2) light chain-containing antibodies account for about 30% of total spore-specific immunoglobulins; and (3) in contrast to all other isotypes, IgG3 antibodies continue to increase over the 3-week period. These findings confirmed the hybridoma analysis that the immune response to *B. subtilis* spores is dominated by a particular set of B cell clones.

EXAMPLE 13

Monoclonal Antibodies to *B. anthracis*

Mice were immunized with a 50:50 mix of heavily irradiated (4×10^6 Gy) *Bacillus anthracis* spores of the Ames and Sterne strains, generated hybridomas, and screened for antibody production by FACS analysis. About 60 hybridomas were selected for further characterization. A similar pattern of reactive antibodies was obtained with some of these panels binding 100% of *B. anthracis* spores. A seen in Figure 6, a representative profile of more than 36 anti-anthrax antibodies which stain all spores but were not at all reactive with *B. subtilis* and *B. thuringiensis* spores. The V_H gene sequences were determined and are shown in Figure 7. Again a

similar conservation in V_H usage was found similar to what was found in antibodies to *B. subtilis*. In this case, one of the two V_H genes is from V_H7183 and the other from the V_HJ558 family predominates. A third V_H gene is from the V_H Q52 family.

EXAMPLE 14

Monoclonal Antibodies to *B. thuringiensis*

A similar strategy was used to isolate and characterize ~100 antibody forming hybridomas which reacted with *B. thuringiensis*. Again the pattern was similar with all reacting with *B. thuringiensis* but not *B. subtilis* or *B. anthracis*. These antibodies were cloned and are sequenced. The discriminatory ability of antibodies is shown in Figures 8 and 9 where it is possible to clearly discriminate three distinct spore staining by fluorescence in a mixture of the three kinds of spores *in vitro* and *in vivo*.

EXAMPLE 15

Discussion

The work presented here disclosed panels of antibodies which are highly specific and can discriminate between spores of the

Bacillus family including the strategically important *B. anthracis* (anthrax). This is the first time such antibodies have been isolated and characterized. The reagents used in the various Divisions of the Armed services for testing were not monoclonal. They were made in sheep and other species against *B. subtilis* and *B. anthracis*.

The antibodies disclosed in the present invention are unique because of several reasons: (1) the spores were not fixed with glutaraldehyde or formalin (which chemically modifies the spores) before immunization; (2) these are monoclonal antibodies made by immunizing and fusing local lymph nodes. Such a procedure has not been used in the past. The few monoclonal antibodies described before have been of the IgM isotypes which are more difficult antibodies to use and are more cross-reactive, i.e., react with spores other than *B. anthracis*, while the monoclonal antibodies disclosed herein are IgG. IgM antibodies of this kind are useless in instruments designed to give positive results for anthrax spores in the field, since such antibodies will also detect harmless spores such as *B. subtilis* which is ubiquitous in the environment.

The amino acid sequences of these anti-spore antibodies were also analyzed, which allows one to design and make smaller peptide molecules which can also bind spores. These will be more rugged molecules than the large antibody molecule and can be used in

other kinds of detectors. Such peptides are totally unique in their binding to *Bacillus* spores.

There are numerous government (services and intelligence), as well as private groups trying to make instruments that are small, portable and highly accurate in their detection of small numbers of potentially lethal spores such as anthrax. The monoclonal antibodies presented here could play a critical role in their instrument development program. Such findings will be significant in detecting air and water containing anthrax spores for civilian and military use.

Any patents or publications mentioned in this specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present examples along with the methods, procedures, treatments, molecules, and specific compounds described herein are presently representative of preferred embodiments, are exemplary,

and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention as defined by the scope of the claims.

WHAT IS CLAIMED IS:

1. A monoclonal antibody highly specific for *Bacillus* spores.

2. The monoclonal antibody of claim 1, wherein said antibody is IgG.

3. The monoclonal antibody of claim 1, wherein said *Bacillus* is selected from the group consisting of *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus subtilis*, and bacilli related to *Bacillus anthracis*.

4. A peptide derived from the monoclonal antibody of claim 1.

5. The peptide of claim 4 binds *Bacillus* spores specifically.

6. A method of preparing a monoclonal antibody highly

specific for *Bacillus* spores, comprising the steps of:

immunizing an animal with said *Bacillus* spores; and

fusing local lymph nodes of said animal.

7. The method of claim 6, wherein said monoclonal antibody is IgG.

8. The method of claim 6, wherein said *Bacillus* is selected from the group consisting of *Bacillus anthracis*, *Bacillus thuringiensis*. *Bacillus subtilis*, and bacilli related to *Bacillus anthracis*.

9. The method of claim 6, wherein said animal is a mouse.

10. A method of detecting *Bacillus* spores in a field sample using a monoclonal antibody specific for said spores, comprising the step of:

contacting said sample with the monoclonal antibody of claim 1.

11. The method of claim 10, wherein said monoclonal antibody is IgG.

12. The method of claim 10, wherein said *Bacillus* is selected from the group consisting of *Bacillus anthracis*, *Bacillus thuringiensis*, *Bacillus subtilis*, and bacilli related to *Bacillus anthracis*.

13. A method of detecting *Bacillus* spores in a field sample, comprising the step of:

contacting said sample with said peptide of claim 4.

14. The method of claim 13, wherein said peptide binds *Bacillus* spores specifically.

Antibodies with different reactivities for spores

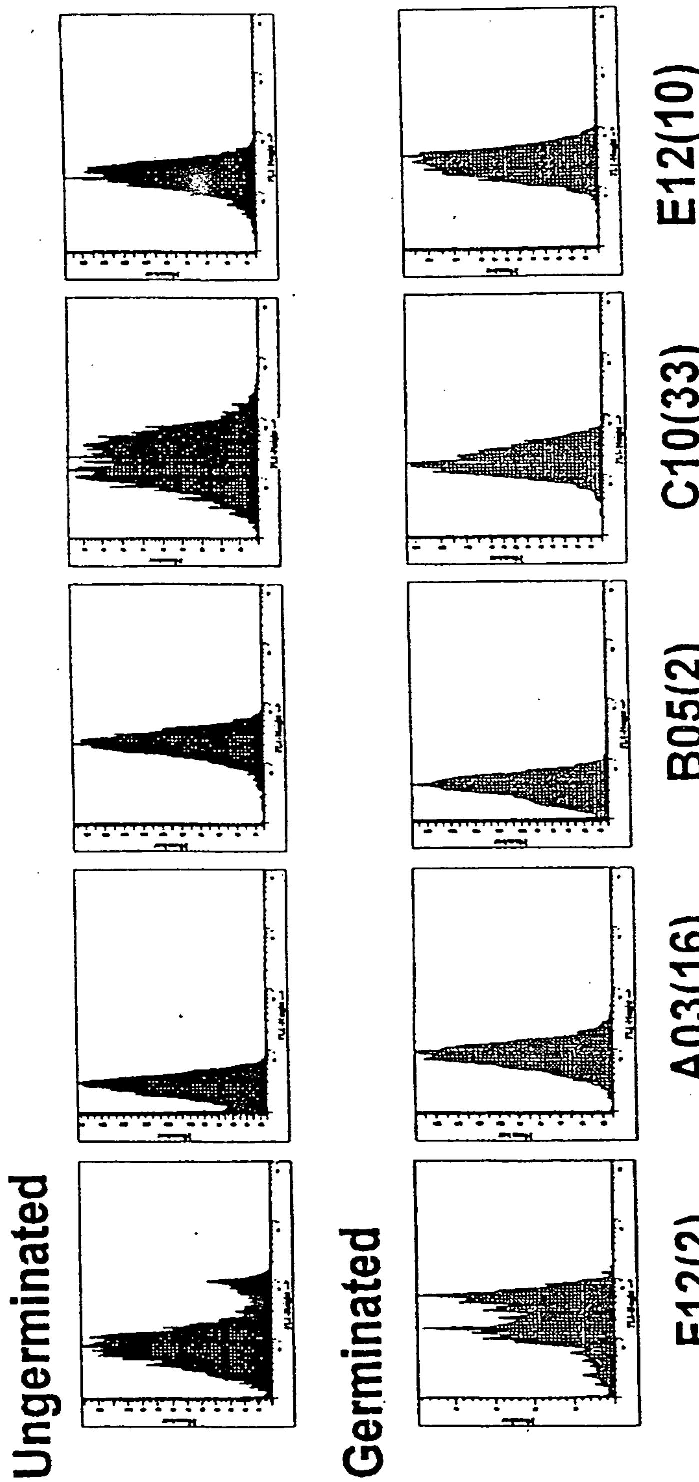


FIG. 1

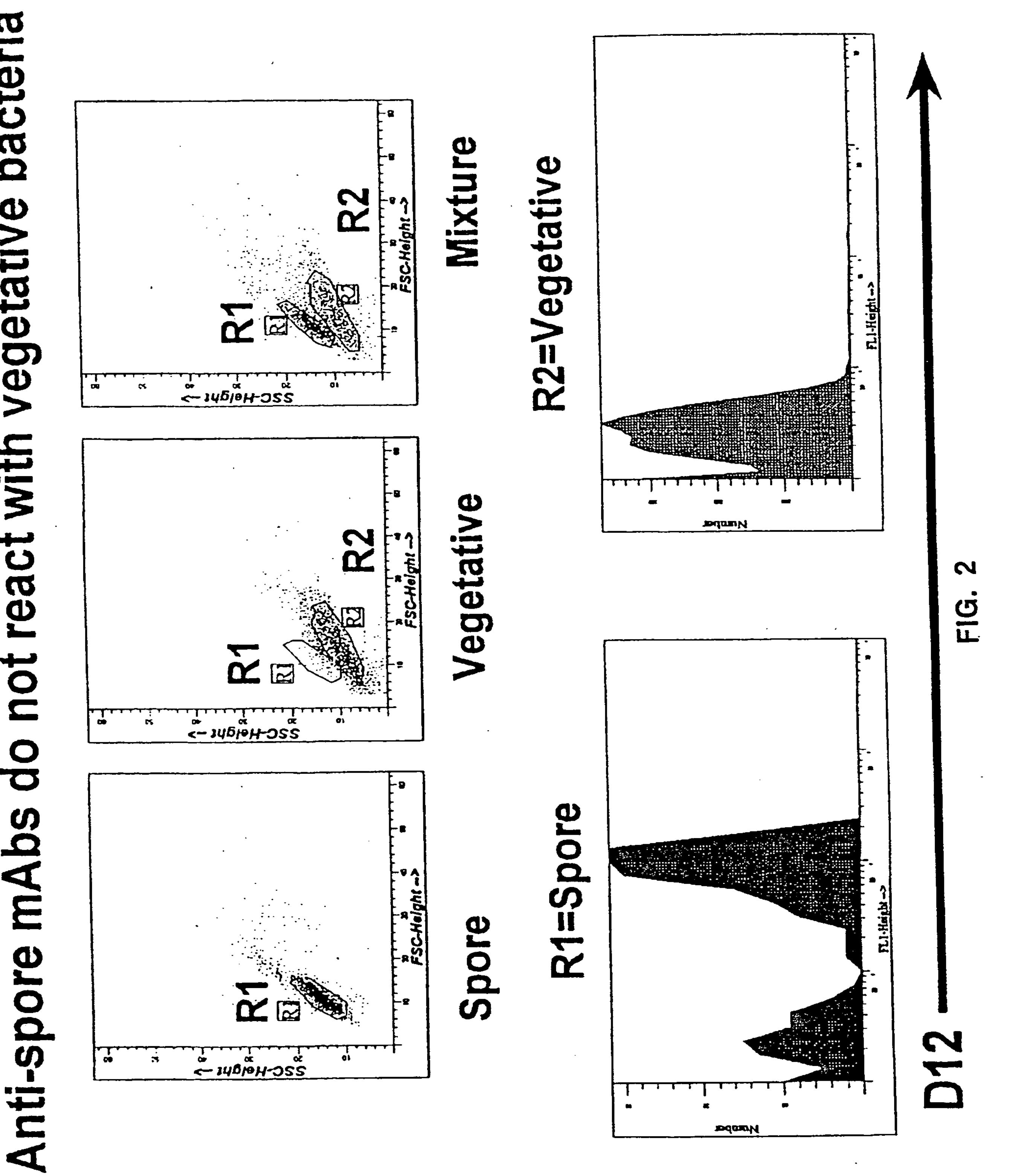
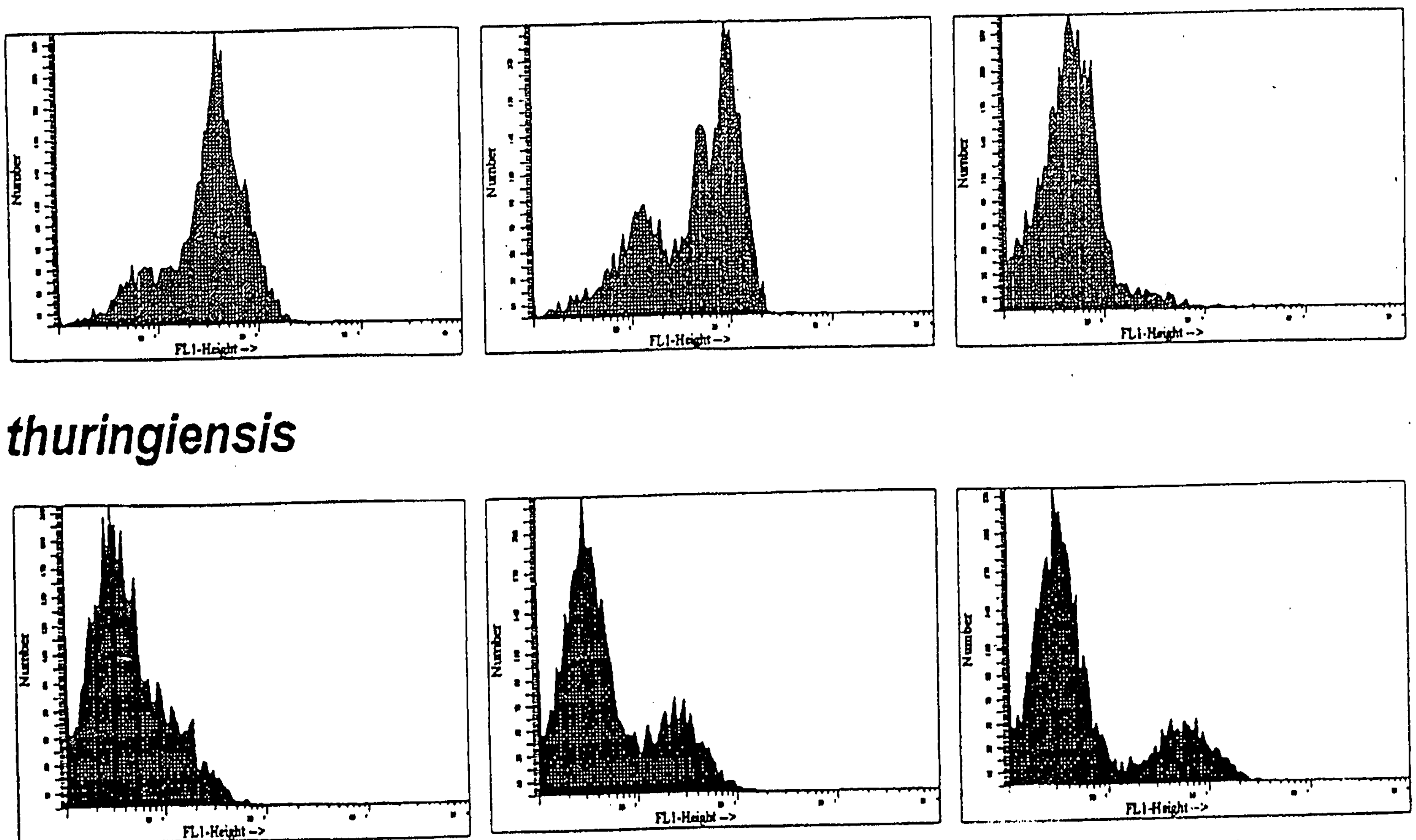

Anti-spore mAbs do not react with vegetative bacteria
R1
R2
SSC-Height -->
FSC-Height -->
Spore
Vegetative
Mixture
R1=Spore
R2=Vegetative
Number
FL1-Height -->
D12

FIG. 2

Most antibodies react specifically with spores of *Bacillus subtilis*

B. subtilis

B. thuringiensis



C11 like most

G6

F11 and other two

FIG. 3

```
g07   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
g04   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
g06   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRLAP[illegible]KGLEWVAYISSGSSTIYYADTVKGRF
d06   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
a07   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
e11   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
a05   1 GGLVQPGGSRKLSCAASGFTFS[illegible]FG[illegible]HWVRQAP[illegible]KGLEWVAYI[illegible]SGSSTIYYADTVKGRF
e07   1 GGLVQPGGSRKLSCAASGFTFS[illegible]FGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
d12   1 GGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
d04   1 GGLVQPGGSRKLSCAASGFTFS[illegible]FGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF
f10   1 GGLVQPGGSRKLSCAASGFTFS[illegible]FGMHWVRQAPEKGLEWVAYISSGSSTIYYADTVKGRF

g07  61 TISRDNPKNTLFLQMTSLRSEDTVMYYCAR....RGTGTRYFDYWGQG[illegible]T[illegible]TVSSE[illegible]Q[illegible]F
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g06  61 TISRDNPKNTLFLQMTSLRS[illegible]DTAMYYCARSGHDYGYSRGYFDVWGAGTTVTVSSE[illegible]Q[illegible]F
d06  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCARSGHYYGYSRGYFDVWGAGTTVTVSSATTTA
a07  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCA[illegible]....WEV[illegible]TRYFDVWGAGTTVTVSSATTTA
 11  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCARYDT..TVV[illegible]RAMDYWGQGT[illegible]VTVSSATTTA
a05  61 TISRDNPKNTLFLQMTSLTSEDTAMYYCARRNC..G.SKRAIDYWGQGT[illegible]VTVSSATTTA
e07  61 TISRDNPKNTLFLQMTNLRSEDTAMYYCARW.....D[illegible]LRTFAYWGQGTLVTVS[illegible]AKTTA
d12  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCARW......LLRAMDYWGQGT[illegible]VTVSSAKTTA
d04  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCARWHYYGTNYVRAMDYWGQGT[illegible]VTVSSAKTTA
f10  61 TISRDNPKNTLFLQMTSLRSEDTAMYYCARHY[illegible]YDEGPHWYFDVWGAGTTVTVSSAKTT[illegible]
```

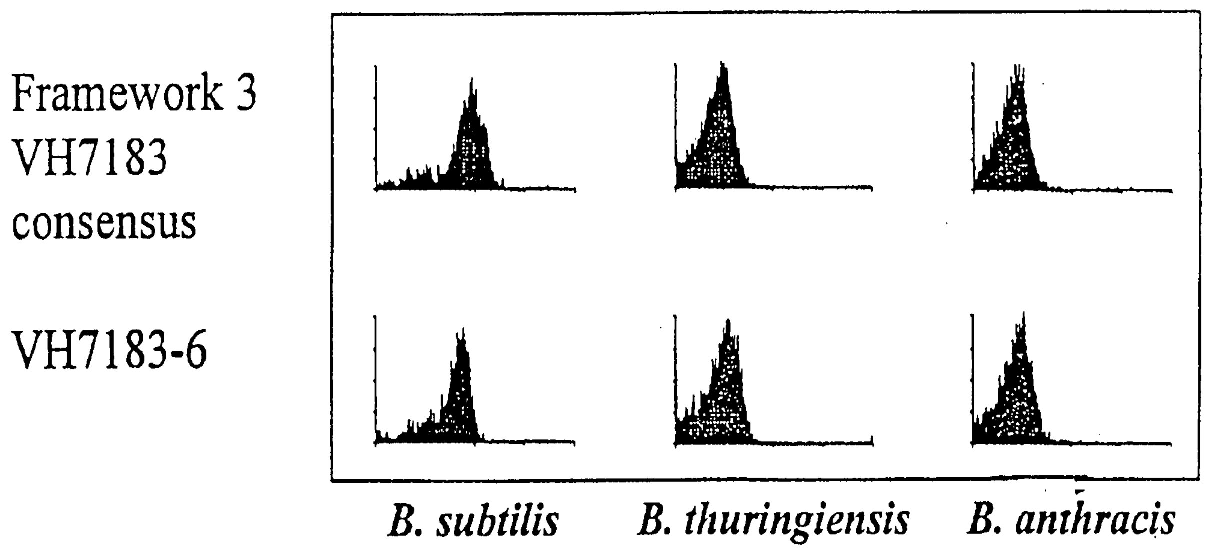
FIG. 4-1

```
g07  117  PNVFPLVSCESPLSDKNLVANGCLDPD~
g04  115  PNVFPLVSCESPLSDKNLVANGCLARD~
g06  121  SNVFPLVSCESPLSDKNLVANGCLDPD~
d06  121  PSVYPLVPGCIDTSGSS.VTLGCLVKAT
a07  117  PSVYPLVPGCSDTSGSS.VTLGCLVKAT
e11  119  PSVYPLVPGCIDTSGSS.VTLGCLVKAT
a05  118  PSVYPLVPGCSDTSGSS.VTLGCLVKAT
e07  116  PSVYPLAPVCGDTTGSS.VTLGCLVKGY
d12  115  PSVYPLAPVCGDTTGSS.VTLRCLVKGY
d04  121  PSVYPLAPGCGDTTGSS.STLGCLVNGY
f10  121  PSVYPLAPGCGDTTGSS.VTLGCLVKGY
```

g07	SEQ ID NO: 7	a05	SEQ ID NO: 13
g04	SEQ ID NO: 8	e07	SEQ ID NO: 14
g06	SEQ ID NO: 9	d12	SEQ ID NO: 15
d06	SEQ ID NO: 10	d04	SEQ ID NO: 16
a07	SEQ ID NO: 11	f10	SEQ ID NO: 17
e11	SEQ ID NO: 12		

FIG. 4-2

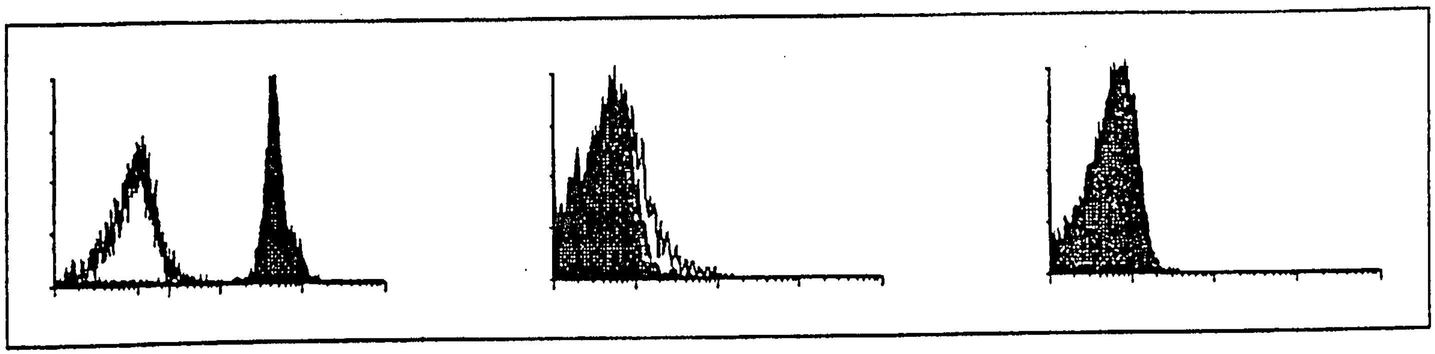
Ab-derived Peptides Specifically Bind *Bacillus subtilis* Spores



VH7183-6	RFTISRDNPKNTLFLQMT	SEQ ID NO: 5
VH7183 consensus	RFTISRDNAKNTLYLQMS	SEQ ID NO: 6

FIG. 5

Anti-*Bacillus anthracis* Ab Specifically Bind *Bacillus anthracis* Spores



B. anthracis *B. subtilis* *B. thuringiensis*

FIG. 6

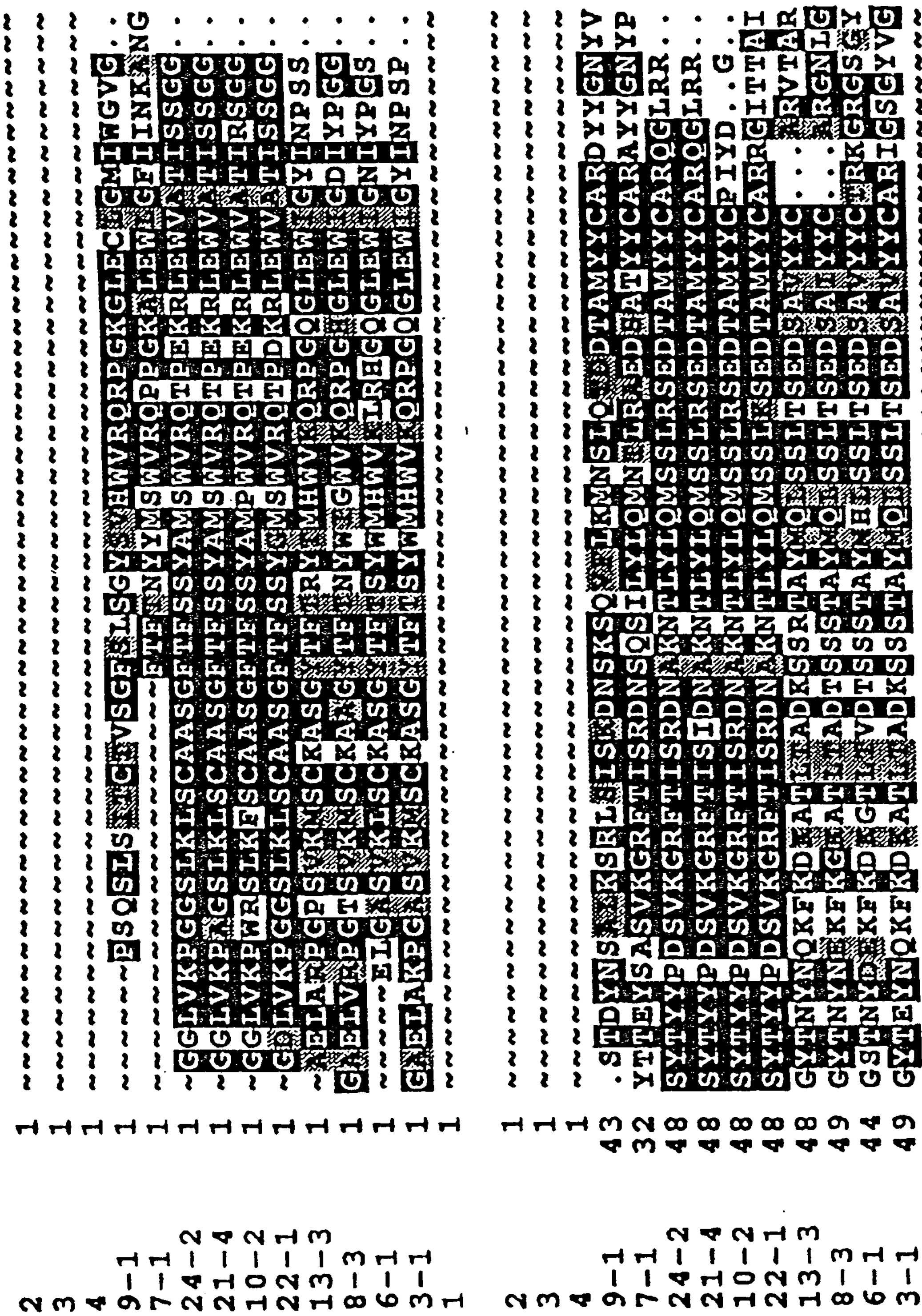


FIG. 7-1

```
2      1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~TYP[illegible]PIR~~~~~~~~~~~~~~~~~~~~~~~~~~
3      1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~TYP[illegible]PFR~~~~~~~~~~~~~~~~~~~~~~~~~~
4      1  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~TYPVPHR~~~~~~~~~~~~~~~~~~~~~~~~~~
9-1   92  WYFDVWGAGTTVTVSSAKTTPPSVYPLAPGCGDTTGSS.VTLGCLVKGY
7-1   82  AWFAYWGQGTLVTVSAAKTTAPSVYQLAPVCGDTTGSS.VTLGCLVKGY
24-2  96  YAMDYWGQGTSVTVSSAKTTPPSVYPLAPGFGDTTGSS.VTLGCLVKGY
21-4  96  VAMDYWGQGTSVTVSSAKTTAPSVYQLAPGFGDTTGSS.VTLGCLVKG~
10-2  94  HAMDYWGQGTSVTVSSATTTAPSVYPLVPGCNDTTGSS.VTLGCLVKGY
22-1  98  YAMDYWGQGTSVTVSSESQSFPNVFPLVSCESPLSDKNLVAMGCLARD~
13-3  95  YAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTTGSS.VTLGCLVKGY
8-3   96  ...DYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTTGSS.VTLGCLVKGY
6-1   94  DAMDYWGQGTSVTVSSAKTTAPSVYPLAPVCGDTTGSS.VTVGCLVKGY
3-1   99  YAMDYWGQGTSVTVSSESQSFPNVFPLVSCESPLSEKNLVAMGCLARD~
1      1  ~~~~~~~~~TSQNVRS~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
```

2	SEQ ID NO: 18	10-2	SEQ ID NO: 25
3	SEQ ID NO: 19	22-1	SEQ ID NO: 26
4	SEQ ID NO: 20	13-3	SEQ ID NO: 27
9-1	SEQ ID NO: 21	8-3	SEQ ID NO: 28
7-1	SEQ ID NO: 22	6-1	SEQ ID NO: 29
24-2	SEQ ID NO: 23	3-1	SEQ ID NO: 30
21-4	SEQ ID NO: 24	1	SEQ ID NO: 31

FIG. 7-2

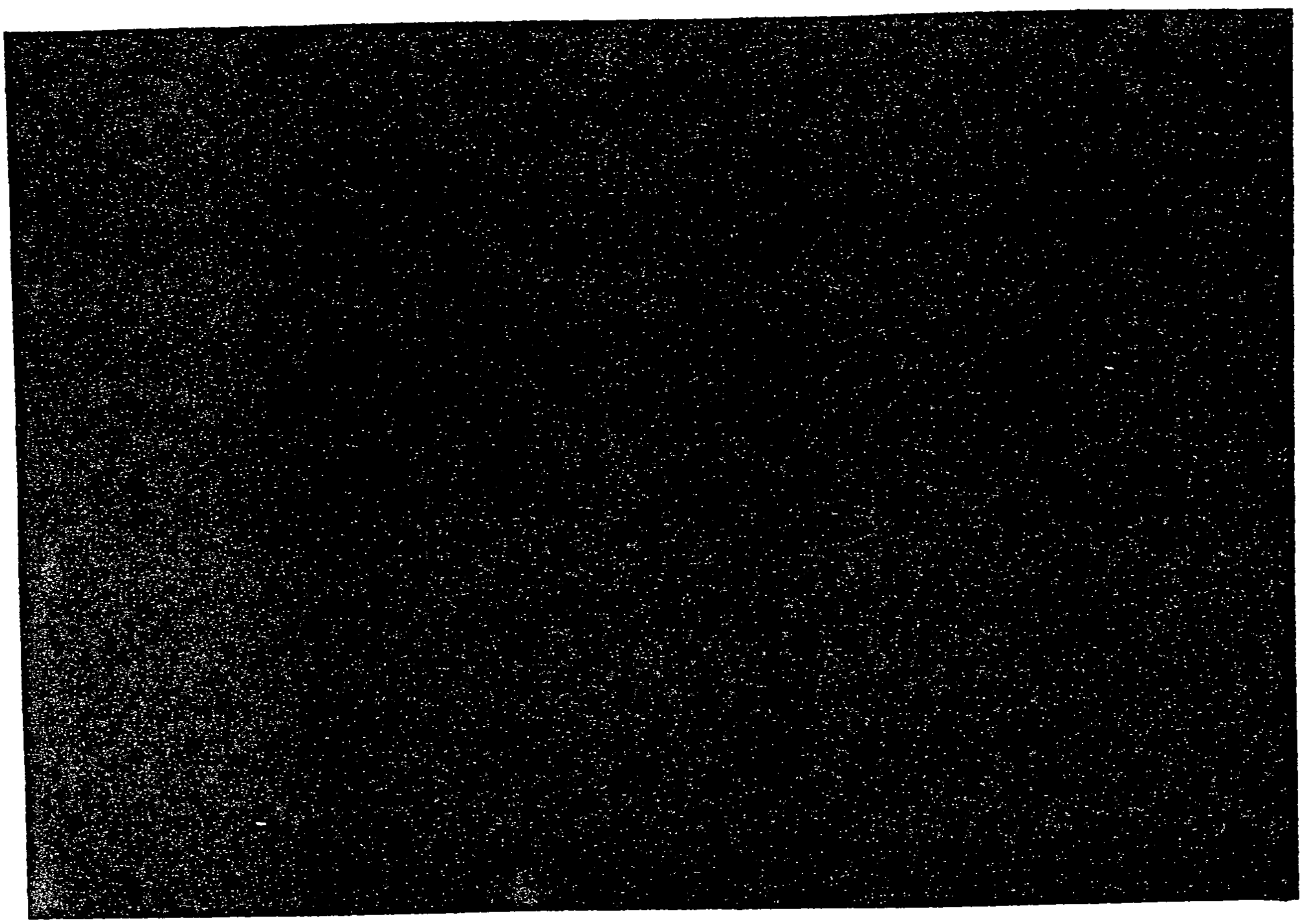


FIG. 8

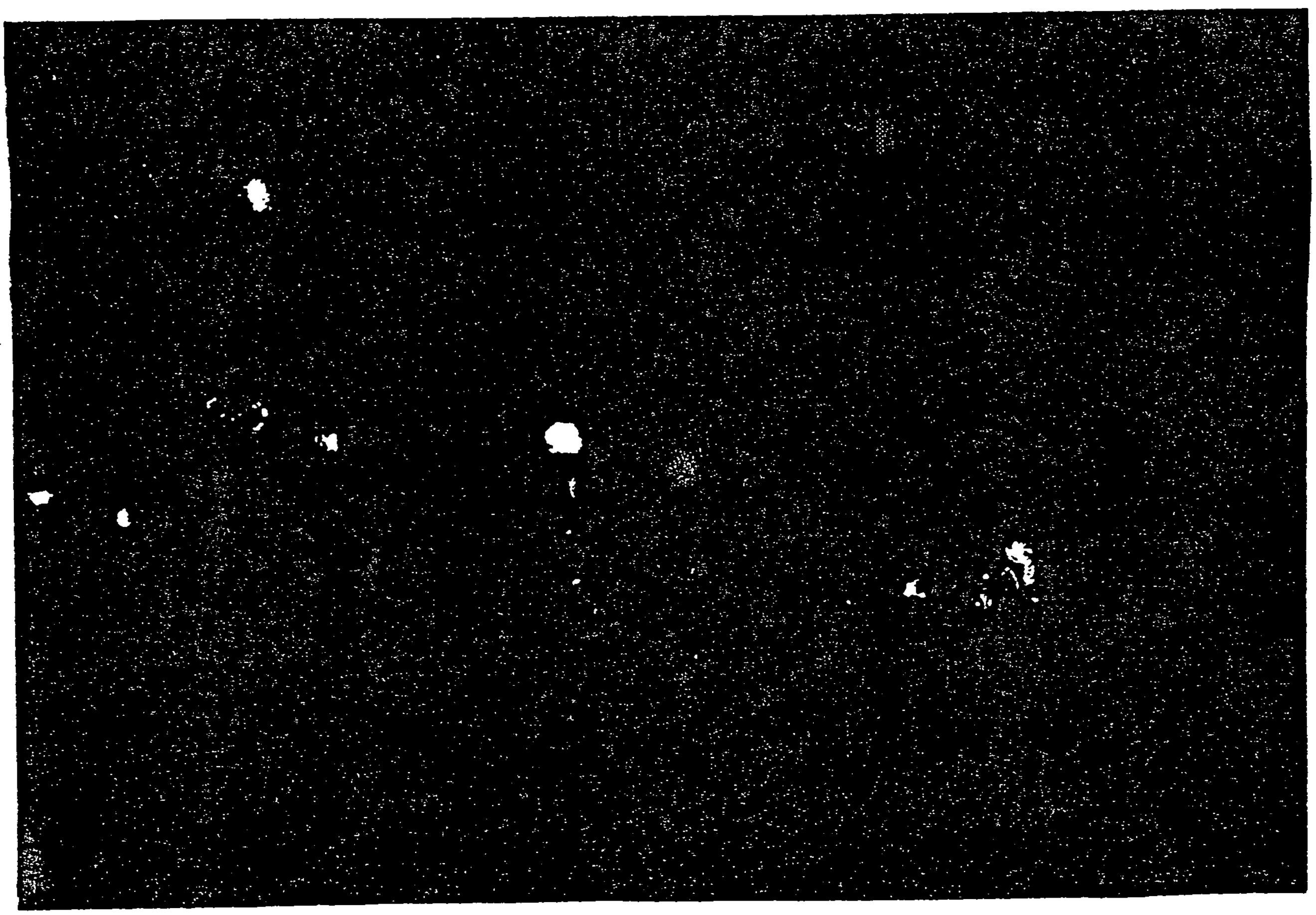


FIG. 9

SEQUENCE LISTING

<110> Kearney, John F.

<120> Monoclonal Antibodies Specific for Anthrax Spores and Peptides Derived from the Antibodies Thereof

<130> D6086PCT

<140>

<141> 1998-04-27

<150> US 09/069,628

<151> 1998-04-29

<160> 31

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<211> 32

<212> DNA

<213> artificial sequence

<220>

<223> Cμ 3' primer used to amplify V_H cDNA

<400> 1

gaagcttata cacagttggt gcagcatcag cc 32

<210> 2

<211> 32

<212> DNA

<213> artificial sequence

<220>

<223> V_H7183-specific primer used to amplify V_H cDNA

<400> 2

cgcgcggccg cgtggagtct gggggaggct ta 32

<210> 3

<211> 30

<212> DNA

<213> artificial sequence

<220>

<223> Cκ 3' primer used to amplify V_K cDNA

<400> 3

gaagcttata cagttggtgc agcatcagcc 30

<210> 4
<211> 30
<212> DNA
<213> artificial sequence
<220>
<223> Cκ 5' primer used to amplify V_{κ} cDNA
<400> 4

gccatggtpr tqlwlmtsac ccagtctcca 30

<210> 5
<211> 18
<212> PRT
<213> artificial sequence
<220>
<223> Peptide Anti-spore VH7183-6 amino acid sequence corresponding to consensus sequence in framework 3 region of *B. subtilis* antibodies.
<400> 5

Arg Phe Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu
5 10 15

Gln Met Thr

<210> 6
<211> 18
<212> PRT
<213> artificial sequence
<220>
<223> Peptide 7183 consensus sequence corresponding to the consensus sequences of the 7183 VH gene family.
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Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu
5 10 15

Gln Met Ser

<210> 7
<211> 143

<212> PRT
<213> artificial sequence
<220>
<223> Hybridoma g07 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 7

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Arg Ser Glu Asp Thr Val Met Tyr Tyr Cys Ala Arg
80 85 90

Arg Gly Thr Gly Thr Arg Tyr Phe Asp Tyr Trp Gly Gln Gly Ala
95 100 105

Thr Leu Thr Val Ser Ser Glu Ser Gln Ser Phe Pro Asn Val Phe
110 115 120

Pro Leu Val Ser Cys Glu Ser Pro Leu Ser Asp Lys Asn Leu Val
125 130 135

Ala Met Gly Cys Leu Asp Pro Asp
140

<210> 8
<211> 141
<212> PRT
<213> artificial sequence

<220>

<223> Hybridoma g04 amino acid sequence of framework three (FR3) region of heavy chain gene.

<400> 8

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Thr
80 85 90

Tyr Arg Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
95 100 105

Thr Val Ser Ser Glu Ser Gln Ser Phe Pro Asn Val Phe Pro Leu
110 115 120

Val Ser Cys Glu Ser Pro Leu Ser Asp Lys Asn Leu Val Ala Met
125 130 135

Gly Cys Leu Ala Arg Asp
140

<210> 9

<211> 147

<212> PRT

<213> artificial sequence

<220>

<223> Hybridoma g06 amino acid sequence of framework three (FR3) region of heavy chain gene.

<400> 9

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Leu Ala Pro Asp Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Arg Ser Asp Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90

Ser Gly His Asp Tyr Gly Tyr Ser Arg Gly Tyr Phe Asp Val Trp
95 100 105

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Glu Ser Gln Ser Phe
110 115 120

Ser Asn Val Phe Pro Leu Val Ser Cys Glu Ser Pro Leu Ser Asp
125 130 135

Lys Asn Leu Val Ala Met Gly Cys Leu Asp Pro Asp
140 145

<210> 10
<211> 147
<212> PRT
<213> artificial sequence
<220>
<223> Hybridoma d06 amino acid sequence of framework

three (FR3) region of heavy chain gene.

<400> 10

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90

Ser Gly His Tyr Tyr Gly Tyr Ser Arg Gly Tyr Phe Asp Val Trp
95 100 105

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala Thr Thr Thr Ala
110 115 120

Pro Ser Val Tyr Pro Leu Val Pro Gly Cys Ile Asp Thr Ser Gly
125 130 135

Ser Ser Val Thr Leu Gly Cys Leu Val Lys Ala Thr
140 145

<210> 11
<211> 143
<212> PRT
<213> artificial sequence
<220>
<223> Hybridoma a07 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 11

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Lys
80 85 90

Trp Glu Val Thr Thr Arg Tyr Phe Asp Val Trp Gly Ala Gly Thr
95 100 105

Thr Val Thr Val Ser Ser Ala Thr Thr Thr Ala Pro Ser Val Tyr
110 115 120

Pro Leu Val Pro Gly Cys Ser Asp Thr Ser Gly Ser Ser Val Thr
125 130 135

Leu Gly Cys Leu Val Lys Ala Thr
140

<210> 12
<211> 145
<212> PRT
<213> artificial sequence
<220>
<223> Hybridoma e11 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 12

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg

20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser

35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe

50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met

65 70 75

Thr Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg

80 85 90

Tyr Asp Thr Thr Val Val Ala Arg Ala Met Asp Tyr Trp Gly Gln

95 100 105

Gly Thr Ser Val Thr Val Ser Ser Ala Thr Thr Thr Ala Pro Ser

110 115 120

Val Tyr Pro Leu Val Pro Gly Cys Ile Asp Thr Ser Gly Ser Ser

125 130 135

Val Thr Leu Gly Cys Leu Val Lys Ala Thr

140 145

<210> 13
<211> 144
<212> PRT
<213> artificial sequence
<220>
<223> Hybridoma a05 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 13

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala

5 10 15

Ala Ser Gly Phe Thr Phe Ser Thr Phe Gly Val His Trp Val Arg

20 25 30

Gln Ala Pro Asp Lys Gly Leu Glu Trp Val Ala Tyr Ile Thr Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Ser Leu Thr Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90

Arg Asn Cys Gly Ser Lys Arg Ala Ile Asp Tyr Trp Gly Gln Gly
95 100 105

Thr Ser Val Thr Val Ser Ser Ala Thr Thr Thr Ala Pro Ser Val
110 115 120

Tyr Pro Leu Val Pro Gly Cys Ser Asp Thr Ser Gly Ser Ser Val
125 130 135

Thr Leu Gly Cys Leu Val Lys Ala Thr
140

<210> 14
<211> 142
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<223> Hybridoma e07 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 14

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Thr Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met
65 70 75

Thr Asn Leu Thr Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90

Trp Asp Ala Leu Arg Thr Phe Ala Tyr Trp Gly Gln Gly Thr Leu
95 100 105

Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro
110 115 120

Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
125 130 135

Gly Cys Leu Val Lys Gly Tyr
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<210> 15
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<213> artificial sequence
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<223> Hybridoma d12 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 15

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Phe Gly Met His Trp Val Arg
20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser
35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met

65 70 75

Thr Ser Leu Thr Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg

80 85 90

Trp Leu Leu Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val

95 100 105

Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu

110 115 120

Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Arg

125 130 135

Cys Leu Val Lys Gly Tyr

140

<210> 16
<211> 147
<212> PRT
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<223> Hybridoma d04 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 16

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala

5 10 15

Ala Ser Gly Phe Thr Phe Ser Thr Phe Gly Met His Trp Val Arg

20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser

35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe

50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met

65 70 75

Thr Ser Leu Thr Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg

80 85 90

Trp His Tyr Tyr Gly Thr Asn Tyr Val Arg Ala Met Asp Tyr Trp

95 100 105

Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Lys Thr Thr Ala

110 115 120

Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly

125 130 135

Ser Ser Leu Thr Leu Gly Cys Leu Val Asn Gly Tyr

140 145

<210> 17
<211> 147
<212> PRT
<213> artificial sequence
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<223> Hybridoma f10 amino acid sequence of framework three (FR3) region of heavy chain gene.
<400> 17

Gly Gly Leu Val Gln Pro Gly Gly Ser Arg Lys Leu Ser Cys Ala

5 10 15

Ala Ser Gly Phe Thr Phe Ser Thr Phe Gly Met His Trp Val Arg

20 25 30

Gln Ala Pro Glu Lys Gly Leu Glu Trp Val Ala Tyr Ile Ser Ser

35 40 45

Gly Ser Ser Thr Ile Tyr Tyr Ala Asp Thr Val Lys Gly Arg Phe

50 55 60

Thr Ile Ser Arg Asp Asn Pro Lys Asn Thr Leu Phe Leu Gln Met

65 70 75

Thr Ser Leu Thr Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg

80 85 90

His Tyr Arg Tyr Asp Glu Gly Pro His Trp Tyr Phe Asp Val Trp

95 100 105

Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr Ser

110 115 120

Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp Thr Thr Gly

125 130 135

Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr

140 145

<210> 18
<211> 7
<212> PRT
<213> artificial sequence
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<223> *B. anthracis* monoclonal antibody 2 VH gene sequence
<400> 18

Thr Tyr Pro Ile Pro Ile Arg

5

<210> 19
<211> 7
<212> PRT
<213> artificial sequence
<220>
<223> *B. anthracis* monoclonal antibody 3 VH gene sequence
<400> 19

Thr Tyr Pro Ile Pro Phe Arg

5

<210> 20
<211> 7

<212> PRT

<213> artificial sequence

<220>

<223> *B. anthracis* monoclonal antibody 4 VH gene sequence

<400> 20

Thr Tyr Pro Val Pro His Arg

5

<210> 21

<211> 139

<212> PRT

<213> artificial sequence

<220>

<223> *B. anthracis* monoclonal antibody 9-1 VH gene sequence

<400> 21

Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser

5 10 15

Leu Ser Gly Tyr Ser Val His Trp Val Arg Gln Arg Pro Gly Lys

20 25 30

Gly Leu Glu Cys Leu Gly Met Ile Trp Gly Val Gly Ser Thr Asp

35 40 45

Tyr Asn Ser Ala Leu Lys Ser Arg Leu Ser Ile Ser Lys Asp Asn

50 55 60

Ser Lys Ser Gln Val Phe Leu Lys Met Asn Ser Leu Gln Thr Asp

65 70 75

Asp Thr Ala Met Tyr Tyr Cys Ala Arg Asp Tyr Tyr Gly Asn Tyr

80 85 90

Val Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val

95 100 105

Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro

110 115 120

Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu

125 130 135

Val Lys Gly Tyr

<210> 22
<211> 129
<212> PRT
<213> artificial sequence
<220>
<223> *B. anthracis* monoclonal antibody 7-1 VH gene sequence
<400> 22

Phe Thr Phe Thr Asn Tyr Tyr Met Ser Trp Val Arg Gln Pro Pro

5 10 15

Gly Lys Ala Leu Glu Trp Leu Gly Phe Ile Ile Asn Lys Ala Asn

20 25 30

Gly Tyr Thr Thr Glu Tyr Ser Ala Ser Val Lys Gly Arg Phe Thr

35 40 45

Ile Ser Arg Asp Asn Ser Gln Ser Ile Leu Tyr Leu Gln Met Asn

50 55 60

Thr Leu Arg Ala Glu Asp Ser Ala Thr Tyr Tyr Cys Ala Arg Ala

65 70 75

Tyr Tyr Gly Asn Tyr Pro Ala Trp Phe Ala Tyr Trp Gly Gln Gly

80 85 90

Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Ala Pro Ser Val

95 100 105

Tyr Gln Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val

110 115 120

Thr Leu Gly Cys Leu Val Lys Gly Tyr

125

<210> 23

<211> 143

<212> PRT

<213> artificial sequence

<220>

<223> *B. anthracis* monoclonal antibody 24-2 VH gene sequence

<400> 23

Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala

5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg

20 25 30

Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser

35 40 45

Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe

50 55 60

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met

65 70 75

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg

80 85 90

Gln Gly Leu Arg Arg Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr

95 100 105

Ser Val Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr

110 115 120

Pro Leu Ala Pro Gly Phe Gly Asp Thr Thr Gly Ser Ser Val Thr

125 130 135

Leu Gly Cys Leu Val Lys Gly Tyr

140

<210> 24
<211> 142
<212> PRT
<213> artificial sequence
<220>
<223> *B. anthracis* monoclonal antibody 21-4 VH gene sequence
<400> 24

Gly Gly Leu Val Lys Pro Ala Gly Ser Leu Lys Leu Ser Cys Ala
5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg
20 25 30

Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser
35 40 45

Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Ile Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
65 70 75

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90

Gln Gly Leu Arg Arg Val Ala Met Asp Tyr Trp Gly Gln Gly Thr
95 100 105

Ser Val Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr
110 115 120

Gln Leu Ala Pro Gly Phe Gly Asp Thr Thr Gly Ser Ser Val Thr
125 130 135

Leu Gly Cys Leu Val Lys Gly
140

<210> 25
<211> 141

<212> PRT

<213> artificial sequence

<220>

<223> *B. anthracis* monoclonal antibody 10-2 VH gene sequence

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5 10 15

Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Pro Trp Val Arg
20 25 30

Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile Arg Ser
35 40 45

Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe
50 55 60

Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
65 70 75

Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys Pro Ile
80 85 90

Tyr Asp Gly His Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
95 100 105

Thr Val Ser Ser Ala Thr Thr Thr Ala Pro Ser Val Tyr Pro Leu
110 115 120

Val Pro Gly Cys Ala Asp Thr Thr Gly Ser Ser Val Thr Leu Gly
125 130 135

Cys Leu Val Lys Gly Tyr
140

<210> 26

<211> 145

<212> PRT

<213> artificial sequence

<220>
<223> *B. anthracis* monoclonal antibody 22-1 VH gene sequence
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5 10 15
Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met Ser Trp Val Arg
20 25 30
Gln Thr Pro Asp Lys Arg Leu Glu Trp Val Ala Thr Ile Ser Ser
35 40 45
Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe
50 55 60
Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln Met
65 70 75
Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Ala Arg
80 85 90
Arg Gly Ile Thr Thr Ala Ile Tyr Ala Met Asp Tyr Trp Gly Gln
95 100 105
Gly Thr Ser Val Thr Val Ser Ser Glu Ser Gln Ser Phe Pro Asn
110 115 120
Val Phe Pro Leu Val Ser Cys Glu Ser Pro Leu Ser Asp Lys Asn
125 130 135
Leu Val Ala Met Gly Cys Leu Ala Arg Asp
140 145

<210> 27
<211> 142
<212> PRT
<213> artificial sequence
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<223> *B. anthracis* monoclonal antibody 13-3 VH gene sequence

<400> 27

Ala Glu Leu Ala Arg Pro Gly Pro Ser Val Lys Met Ser Cys Lys
5 10 15

Ala Ser Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Lys
20 25 30

Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro
35 40 45

Ser Ser Gly Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Lys Ala
50 55 60

Thr Leu Thr Ala Asp Lys Ser Ser Arg Thr Ala Tyr Met Gln Leu
65 70 75

Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg
80 85 90

Val Thr Ala Arg Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser
95 100 105

Val Thr Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro
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Leu Ala Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu
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Gly Cys Leu Val Lys Gly Tyr
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Lys Ala Ala Gly Tyr Thr Phe Thr Asn Tyr Trp Ile Gly Trp Val
20 25 30

Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile Gly Asp Ile Tyr
35 40 45

Pro Gly Gly Gly Tyr Thr Asn Tyr Asn Glu Lys Phe Lys Gly Lys
50 55 60

Ala Thr Leu Thr Ala Asp Thr Ser Ser Ser Thr Ala Tyr Met Gln
65 70 75

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys Ala
80 85 90

Arg Gly Asn Leu Gly Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
95 100 105

Val Ser Ser Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro Leu Ala
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Pro Val Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys
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Leu Val Lys Gly Tyr
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35 40 45
Thr Asn Tyr Asp Glu Lys Phe Lys Asp Lys Gly Thr Leu Thr Val
50 55 60
Asp Thr Ser Ser Ser Thr Ala Tyr Met His Leu Ser Ser Leu Thr
65 70 75
Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Lys Gly Arg Gly
80 85 90
Ser Gly Tyr Asp Thr Met Asp Tyr Trp Gly Gln Gly Thr Ser Val
95 100 105
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Cys Leu Val Lys Gly Tyr
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Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met His Trp Val
20 25 30

Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Asn Ile Asn
35 40 45

Pro Ser Pro Gly Tyr Thr Glu Tyr Asn Gln Lys Phe Lys Asp Lys
50 55 60

Gly Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln
65 70 75

Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala
80 85 90

Arg Ile Gly Ser Gly Tyr Val Gly Tyr Ala Met Asp Tyr Trp Gly
95 100 105

Gln Gly Thr Ser Val Thr Val Ser Ser Glu Ser Gln Ser Phe Pro
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Asn Val Phe Pro Leu Val Ser Cys Glu Ser Pro Leu Ser Glu Lys
125 130 135

Gln Leu Val Ala Met Gly Cys Leu Ala Arg Asp
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Thr Ser Gln Asn Val Arg Thr
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09122

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/06; A61K 35/00, 39/102 37/00
US CL :424/ 93.462, 200.1, 290; 435/ 69.7, 340; 514/2; 800/205
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
MANUAL OF MICONIOLOGY AND BIOTECHNOLOGY, SPORES, SPORE ANTIGENS, INFECTION AND IMMUNOLOGY, MICROB. PATHOG.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,254,799 A (DE GREVE et al.) 19 October 1993, col. 15-18.	1-5
Y,P	US 5,753,222 A (MARRONE et al.) 19 May 1998, col. 1 lines 37-49.	1-5
A	US 5,686,113 A (SPEAKER et al.) 11 November 1997, col. 4-8, col. 12-13, col. 17 lines 36-48.	1-5
Y	WALKER et al. Immunology of Sores and Sporeforms, Anitgens of the Spore and Location of Spore and Vegatative Anitgens With Labeled Anitobdies. 1972, Vol. 5, pages 321-337, see entire document.	1-5 and 10- 14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 JULY 1999	02 SEP 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer D. Lawrence for JA-NA HINES Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09122

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LITTLe et al. Comparative Efficacy of Bacillus-Anthracis Live Spore Vaccine and Protective Antigen vaccine Against Anthrax in the Guinea-Pig. Infect. and Immun. May 1986, Vol. 53, No. 2, pages 509-512.	1-14

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/ 93.462, 200.1, 290
435/ 69.7, 340
514/ 2
800/205